DIFFUSION MEASUREMENTS BY NUCLEAR MAGNETIC RESONANCE (NMR)

Before reading the following script it is recommended first obtain basic knowledge about Nuclear Magnetic Resonance (NMR). See, for example, refs. [1, 2] or in the NMR script in the web page of the Institut für Physikalische Chemie ("Grundlagen der NMR-Spektroskopie" in http://www.uni-muenster.de/Chemie.pc/studieren/app_p.html).

1 Introduction

Diffusion is the random translational (or Brownian) motion of molecules or ions that is driven by internal thermal energy. Translational diffusion is the basic mechanism by which molecules are distributed in space and is considered to play a central role in any chemical reaction since the reacting species have to collide before the reaction can occur. Classically, the mathematics of the diffusion process (i.e. Fick's first and second law of diffusion) has been worked out for systems in which an initial concentration gradient is established. The Fick's first law postulate that the flux of material across a given plane is proportional to the concentration gradient across the plane,

$$J = -D\frac{\partial C(x,t)}{\partial x}$$

where J is the flux, D is the diffusion constant for the material that is diffusing in the specific solvent, and $\frac{\partial C}{\partial x}$ is the concentration gradient. The diffusion constant of a material is expressed in units of length^2/time. The negative sign of the right side of the equation indicates that the impurities are flowing in the direction of lower concentration. Fick's First Law does not consider the fact that the gradient and local concentration of the impurities in a material decreases with an increase in time, an aspect that's important to diffusion processes. On the other hand, Fick's Second Law states that the change in the concentration over time is equal to the change in local diffusion flux, or

$$\frac{\partial C(x,t)}{\partial t} = -\frac{\partial J}{\partial x}$$

or, from Fick's First Law,

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2} \tag{1}$$

where we suppose that the diffusion coefficient is independent of position. In a isotropic system, without thermal or concentration gradients, the average molecule displacement in all three directions is zero but the mean square displacement during a time t is non-zero and is given by

$$\langle r^2 \rangle = 6Dt.$$

Following the Stoke's law, the force needed to move a small sphere of radius R through a continuous medium of viscosity η with a velocity V is

$$F = 6\pi\eta RV. \tag{2}$$

The Stokes-Einstein equation describes the way that diffusion increases in proportion to temperature, and is inversely proportional to the frictional force experienced by a molecule

$$D = \frac{k_B T}{f} \tag{3}$$

where f is given by the Stoke's law, $6\pi\eta R$. Combining eqs. (2) and (3) leads to the well known relation

$$D = \frac{k_B T}{6\pi\eta R}. \tag{4}$$

Typical self diffusion coefficients in liquids at room temperature range from 10^{-9} m^{2}s^{-1} (small molecules in non viscous solution) to 10^{-12} m^{2}s^{-1} (high polymers in solution).

Nuclear Magnetic Resonance (NMR) technique is suitable to study static properties of the matter (ie. structure) and dynamic properties like self-diffusion, flow and relaxation. In the very early story of NMR, in his pioneering work on spin echos, Hahn [3] pointed out that the echo amplitude would be influenced by molecular diffusion (Brownian motion) because of the fluctuations of the local magnetic field. The PFG NMR method was first theoretically and experimentally demonstrated by Stejskal and Tanner [4] and is still in its original form one of the main NMR methods for obtaining dynamic information, such as the self-diffusion coefficient. Self-diffusion data provide detailed information about molecular organization and phase structure. Self-diffusion rates are quite sensitive to structural changes and to binding and association phenomena. Experimental self-diffusion values are directly related to molecular displacement. Apart form the pioneering works mentioned above, a number of reviews articles and books on the basic methodology have appeared [5, 6, 7, 8].

2 Hahn echo

Before starting with the diffusion experiments and, in order to see clearly the effect of the pulse sequences employed to measure diffusion, we will discuss the spin echo sequence (SE). This sequence was introduced by Hahn and is shown in figure 1.

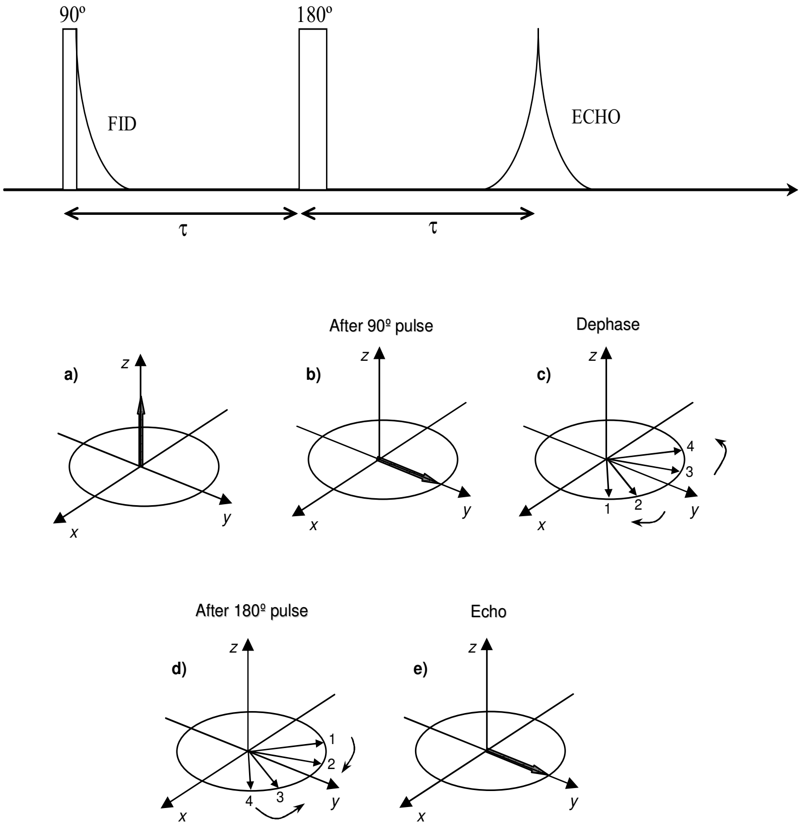

Figure 1: Spin echo (SE) pulse sequence.

Iniatially the magnetization is parallel to the external field (fig. 1a). A 90^o pulse is then applied along the x direction so that the net magnetization now lies in the xy plane, in the y axis (fig. 1b). During the period of time following the removal the RF pulse, each spin experiencing a slight variation in magnetic field begin to fan out slowly or "dephase" as shown in fig. 1c. The variations in the magnetic field come from both transverse relaxation due to T_2 (spin-spin interaction) and inhomogeneities in the external field.

The importance of the spin echo experiment is that the effects of the inhomogeneities are made reversible. At a time a 180^o pulse is applied along the y direction as shown in fig. 1d. The spins are therefore rotated by 180^o around the y axis thereby remaining in the xy plane. As a result of the inverted relative positions, and because each spin continues to precess with its former frequency, all spins will be perfectly reclustered at time 2τ forming a echo (fig. 1e).

3 Basis of Pulse Field Gradient (PFG) NMR

All NMR theory needed for explaining the effects of magnetic field gradients on nuclear spins has the Larmor equation as its origin

$$\omega_o = \gamma B_o \tag{5}$$

where γ is the gyromagnetic ratio and B_o is the strength of the external static magnetic field which is homogeneous over all space. In the presence of an additional magnetic field B_z, then the effective frequency is

$$\omega_{eff} = \omega_o + \gamma B_z. \tag{6}$$

If we suppose that the magnetic field B_z gradient is constant, $g = \frac{dB_z}{dz}$, the effective frequency is

$$\omega_{eff} = \omega_o + \gamma g z. \tag{7}$$

In fig. 2 the effective Larmor frequency is shown for different spins in a sample.

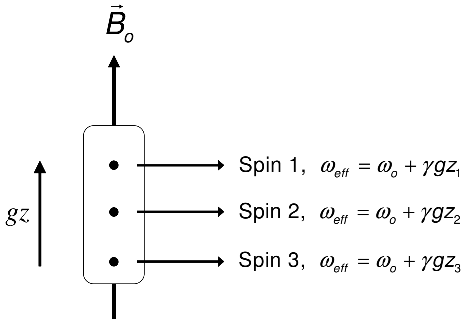

Figure 2: The spin frequency in a inhomogeneous field which varies linearly in one direction over the sample volume. The total magnetic field is the sum of the homogeneous static magnetic field and the applied field gradient.

Measurements of diffusion commonly employ a spin echo pulse sequence as described before. The most common PFG NMR diffusion experiment is a spin echo pulse sequence with pulse magnetic field gradient (PFG SE). Monitoring of the self-diffusion in a sample is accomplished by the application of the magnetic field gradient during the dephasing and rephasing periods. In figure 3 a schematic picture of the pulse sequence is displayed. Between the two RF pulses and after 180^o pulse a field gradient of length δ and strength g are applied.

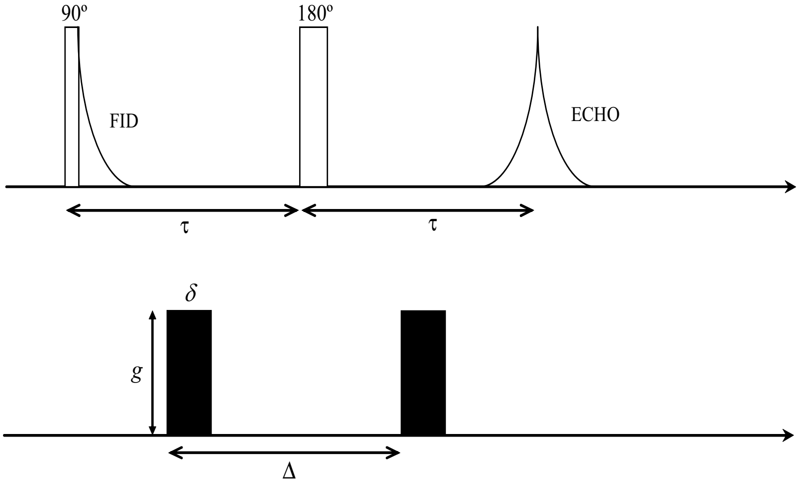

Figure 3: The pulse field gradient spin echo (PFG SE) sequence. The echo appears at 2τ. Sandwiched between the two RF pulses and after the 180^o pulse are two pulse magnetic field gradient of length δ and strength g.

These gradients cause the spin in different positions in the sample to precess differently, thereby enhancing the dephasing process. If the spins maintain their positions throughout the experiment, they will refocus completely into a spin echo by the pulse sequence. On the other hand, if they change position during the experiment, their precession rates will also change, and the refocusing will be incomplete, resulting in a decrease in the intensity of the spin echo. After the first pulse gradient, the phase shift is

$$\phi_1 = \underbrace{\phi_o}_{\substack{\text{static}\\ \text{field}}} + \underbrace{\int \gamma g z \, dt}_{\substack{\text{apllied}\\ \text{gradient}}} = \phi_o + \gamma g z_1 \delta \tag{8}$$

where z_1 is the position of the spin. After the second pulse

$$\phi_2 = \phi_o + \gamma g z_1 \delta - \gamma g z_2 \delta \tag{9}$$

$$= \quad \phi_o + \gamma g \delta (z_1 - z_2). \tag{10}$$

Note that the effect of applying a refocusing radio frequency pulse (180^o) is simply a change in the sign of the gradient. For a quantitative treatment we have to calculate the effect of the pulse gradient in the tranverse magnetization. There exist several approaches of theoretically describing the signal attenuation in PFG NMR diffusion experiments. Here, a brief classical derivation of the attenuation of the spin echo due the translational diffusion will be presented in terms of the macroscopic nuclear magnetization $\vec{M}(\vec{r},t) = M_x\hat{e}_x + M_y\hat{e}_y + M_z\hat{e}_z$ and the Bloch equations [2].

Combining the Bloch equations with Fick's second law gives,

$$\frac{\partial \vec{M}(\vec{r},t)}{\partial t} = \gamma \vec{M} \times \vec{B} - \frac{M_x\hat{e}_x + M_y\hat{e}_y}{T_2} - \frac{M_z - M_o}{T_1}\hat{e}_z + D\nabla \cdot \nabla \vec{M} \tag{11}$$

where T_1 and T_2 are the longitudinal and tranverse relaxation times respectively, M_o is the equilibrium magnetization and $\nabla \cdot \nabla$ is the Laplace operator

$$\nabla \cdot \nabla \equiv \nabla^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}.$$

Now, lets assume that the external magnetic field is given by and the total field

$$B_z = B_o + \vec{g}.\vec{r} \tag{12}$$

where the magnetic field gradient, $\vec{g}$, is given by

$$\vec{g} = \frac{\partial B_z}{\partial x}\hat{e}_x + \frac{\partial B_z}{\partial y}\hat{e}_y + \frac{\partial B_z}{\partial z}\hat{e}_z.$$

From the mathematical point of view, the transverse magnetization is now conveniently written as

$$\Psi = (M_x + iM_y)e^{-i\omega_o t + t/T_2} \tag{13}$$

to include the relaxation term (T_2). Insertion of eq. (13) into eq. (11) gives the equation

$$\frac{\partial \Psi}{\partial t} = -i\gamma \vec{g}.\vec{r}\Psi + D\nabla \cdot \nabla \vec{M} \tag{14}$$

and by setting

$$\Psi(\vec{r},t) = \Psi(t)e^{-i\gamma \vec{r}.\int_0^t \vec{g}(t')dt'} \tag{15}$$

one obtain

$$\frac{\partial \Psi}{\partial t} = -D\gamma^2 \left(\int_0^t \vec{g}(t')dt' \right)^2 \Psi(t). \tag{16}$$

The integral take into account the effect of the dephasing due to the application of the field gradient pulses (see eq. (9)). The solution of eq. (13) can be written as

$$\frac{\partial \Psi}{\partial t} = \Psi(0) \exp \left[-D\gamma^2 \int_0^t \left(\int_0^{t'} \vec{g}(t'')dt'' \right)^2 dt' \right]. \tag{17}$$

The last equation can be rewritten as

$$\ln \left[\frac{S(t)}{S(0)} \right] = -D\gamma^2 \int_0^t \left(\int_0^{t'} \vec{g}(t'')dt'' \right)^2 dt' \tag{18}$$

where $S(t)$ and $S(0)$ represent the signal intensity at time t with and without magnetic field gradients, respectively. In other words, $S(0)$ contains the effect of the relaxation only. Equation (17) is important for the understanding of diffusion (or spin displacements) measurements with NMR. By introducing a well defined magnetic field (in amplitude and spatial variation) in addition to the static magnetic field the positions of the spin can be labeled, albeit indirectly, through their Larmor frequency (see eq. (6)). For the SE sequence, following eq. (18), the signal is

$$S(2\tau) = S(0) \underbrace{\exp\left(-\frac{2\tau}{T_2}\right)}_{\substack{\text{attenuation due}\\ \text{to relaxation}}} \underbrace{\exp\left[-D\gamma^2 g^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right)\right]}_{\substack{\text{attenuation due}\\ \text{to diffusion}}} \tag{19}$$

where the effect of T_2 relaxation is included.

Another useful sequence is a combination of a pulse magnetic field gradient and a sequence called stimulated echo (PFG STE) as shown in figure 4. In this case, the corresponding equation for the signal is (from eq. (18))

$$S(2\tau) = \frac{1}{2} S(0) \underbrace{\exp\left(-\frac{T}{T_1}\right) \exp\left(-\frac{2\tau}{T_2}\right)}_{\substack{\text{attenuation due}\\ \text{to relaxation}}} \underbrace{\exp\left[-D\gamma^2 g^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right)\right]}_{\substack{\text{attenuation due}\\ \text{to diffusion}}}. \tag{20}$$

The advantage with the stimulated pulse sequence is that the effective time for T_2 relaxation, 2τ, can be made short. After the 2^{nd} 90^o pulse and during the time T, the magnetization is along the $-z$, therefore there is no T_2 relaxation. The reason to for making this time short is that T_2 is often short for macromolecules and consequently most of the signal has disappeared at the time of the echo. T_1 on the other hand is often considerably longer.

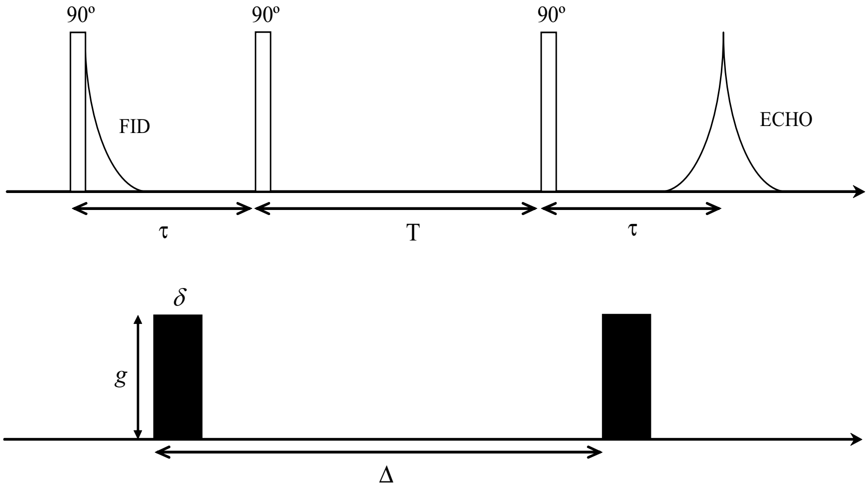

Figure 4: The pulse field gradient stimulated echo sequence (STE). This is often used in macromolecular systems with a short T_2.

4 Some comments about the pulse sequences

The choice of the pulse sequence to use in a PFG NMR diffusion experiment depends on several factors. Generally it is of great importance to consider the relaxation times T_1 and T_2 of the nuclei under study. Secondly, one has to consider the practical problems associated with the application of strong magnetic field gradients, since these cause disturbance in the NMR spectrometer. This latter problem often demands along waiting time after each applied gradient pulse, which sets a limit on the time intervals between RF pulses. The interplay between the relaxation rates and the demand for sufficiently long time intervals between pulses put limits to the accessible diffusion coefficients to be measured. If we compare the two sequences described above, the PFG STE pulse sequence should be used when $T_1 \gg T_2$ for the investigated system. This can be understood by realizing that during the delay T the spins only experience T_1 relaxation. So, in order to optimize the PFG STE pulse sequence, the time when the spin experience T_2 relaxation should be as short as possible. It is important to note that half of the intensity is lost when using PFG STE pulse sequence (see the factor $\frac{1}{2}$ in eq. (20)). In other words, PFG STE pulse sequence should be used for the situation discussed above.

5 Typical diffusion measurements

A typical experiment to measure a self-diffusion coefficient consist in acquire a set of spectra employing to different values of the field gradient strength, g, or the length of the gradient pulse, δ, while the other parameters are held constant (see fig. 5). By plotting the intensity of the echo (I) versus g or δ, it is possible to obtain the self-diffusion coefficient from the decay of the echo intensity (see fig. 6).

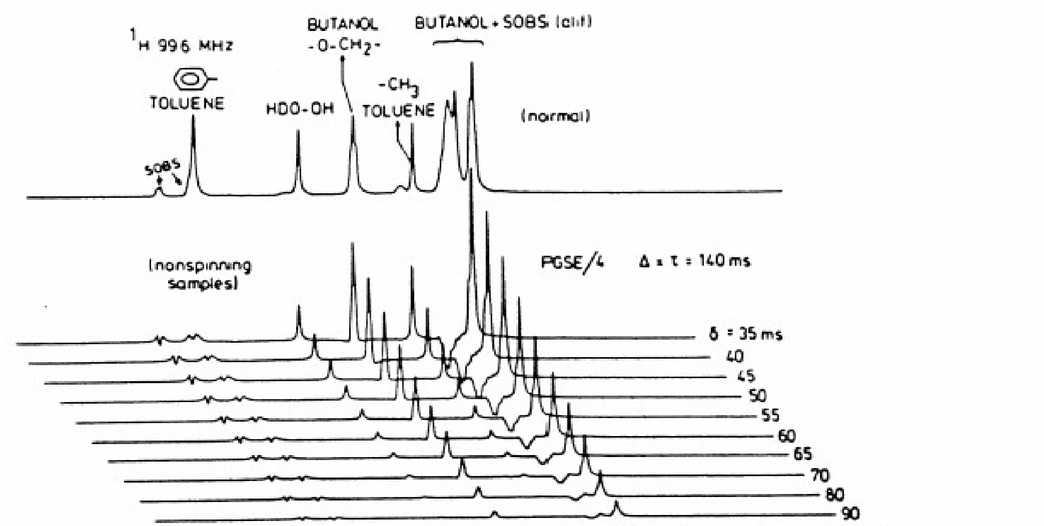

Figure 5: A ^{1}H FT-PFG-NMR stack plot obtained at 99.6 MHz for a microemulsion sample containing sodium actylbenzenesulfonate (SOBS), butanol, toluene and water (D_2O)

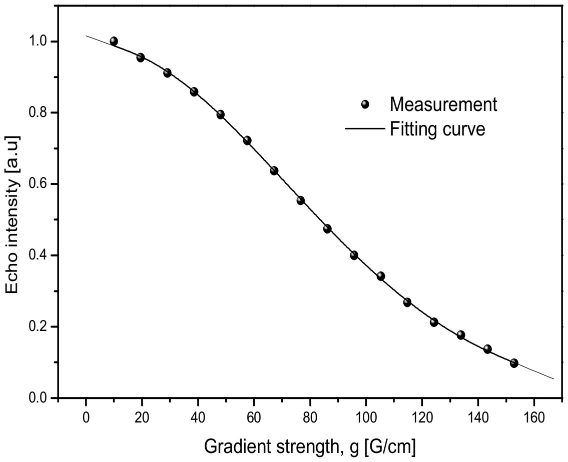

Figure 6: Plot of the echo intensity (I) *vs.* g. From the fit using eq. (20) the diffusion constant is obtained.

References

[1] Levitt, M. H., Spin dynamics, John Wiley and Sons, 2002.

[2] Slichter, C. P., Principles of Magnetic Reonance, Springer-Verlag, Berlin, 1996.

[3] E. L. Hahn, *Phys. Rev.*, **80**, 580 (1950).

[4] Stejkal, E. O and Tanner, J. E., *J. Chem Phys.*, **42**, 288 (1965).

[5] Callaghan, P. T., Principles of Nuclear Magnetic Resonance, Oxford University Press: Oxford, 1991.

[6] R. Kimmich, NMR: Tomography, Diffusometry, Relaxometry, Springer, Berlin, 1997.

[7] Stilbs, P., *Prog. NMR Spectrosc.*, **19**, 1 (1987).

[8] Kärger J., Pfeifer, H. and Heink, W., *Adv. Mag, Res.*, **12**, 1 (1988).

Experimental work

The aims of this laboratory experience are:

1. To learn some practical aspects of NMR, data acquisition and processing in a Bruker DMX 400 spectrometer.
2. set up an experiment to measure the diffusion coefficient using PFG NMR technique.

The proposed studies to be carried out are:

Diffusion of small molecules: A sample containing a mixture of different molecules in solution will be measured in order to distinguish the mobility of each compound in the sample.

Surfactant: Surfactants constitute a vast extensive area of research covering both basic and industrial applications. The idea is to measure the dependence of the diffusion coefficient with the concentration of surfactant in different samples to see the aggregation.

Some basic commands and procedures for the DMX 400 NMR spectrometer

How to read the commands:

italic: commands to type in command line

CAPITAL: commands from pull down menu in xwinnmr

*icon: icon to click on

Acquisition parameters

aq	acquisition time, set to > T2
td	number of points in time domain
sw (swh)	spectral width in ppm or Hz
dw	dwell time (=time between two data points in time domain) note that *dw*, *sw*, *td* and *aq* are interdependent parameters!
p1	pulse length 90° pulse (p2: 180° pulse)
ns	number of scans 1 or multiple of phase cycling list
d1	relaxation delay, set to 5×T1
dn	other delay times
de	dead time, minimum 4.5 μs
rg	receiver gain
acqu	acquisition window
zg	run

Diffusion experiments: acquisition

Create a 2D dataset. Use the tool *diff* to set up the experiment. *diff* will write the necessary lists, au and grad-programs for some standard diffusion experiments. After using diff, don't change parameters in *ased* or *eda* any more, this might lead to software bugs.

Parameters to set in diff window:

- PGSE/PGSTE: Choose: Pulse Gradient Spin Echo or Pulse Gradient Stimulated Echo.
- Exp./Setup: Setup runs with TD1=1 as a pseudo 1D experiment for testing and tuning.
- Linear/sqrt.: Sets the gradient value list linearly or as square root function in the range between min and max.
- Gradient strength: Set min (Grad start) and max value. The maximum possible max value is 1200 G/cm.
- Number of gradients: Number of gradient values, typical: 16
- Grad pulse duration (δ): default: 1 ms. This is very important!!!! Do not use larger values!!!!
- DELTA (Δ): diffusion time (=gradient spacing), reasonable values: 20 ms to 500 ms, multiple of 20 ms.
- grad ramp time: default 0.15 ms.
- Grad stabilization time: waiting time until blanking closes again, default 5 ms. Ramp Y/N
- Number of scans: = NS
- Number of repetitions: 1
- Number of dummy scans, performed only before 1^{st} gradient
- Repetition time: = d1 OBS: in ms!! Echo time (in STE only): time between 1^{st} and the 2^{nd} 90^o pulses. Spoil grad (in STE only): spoil grad after 2^{nd} RF pulse.

*save: use when finished to save the newly created files. (Note that diff always refers to the experiment that was active when the diff window was opened, and saves to this exp.file! When changing experiments, you have to close and re-open diff!)

*quit: close diff without saving changes

*ok = save and quit

Diffusion: Processing

- *xf2*
- *phase: go to 2D window for phasing
- *row: select single spectrum for phasing with middle mouse button
- *(mov) 1: move selected spectrum to display 1
- *ph0: phasing with left mouse button, when finished
- *return: leave phase window with 'save and return'
- *abs2*: to perform baseline correction
- *utilities/*def-pts: define peaks with mouse
- *integrate: if area evaluation required, define peak integrals for evaluation
- *return: leave integration window with save and return

- ANALYSIS / RELAXATION. Always go back to relaxation menu directly after basl/integr, because here the 1D spectral display acts as a subroutine of the relaxation window!
- *edt1*: chose fit type intensity/area
- *pd*: reads data from all spectra
- Do simfit